The Biosynthesis of Ristocetin

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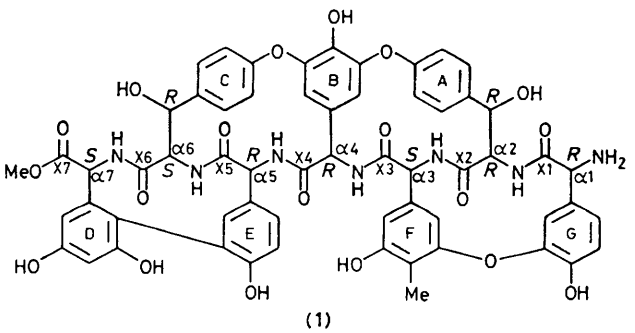
In the biosynthesis of ristocetin by *Nocardia lurida* it has been shown that *m,m'*-dihydroxyphenylglycine and *p*-methyl-*m,m'*-dihydroxyphenylglycine can be derived from acetic acid, whereas *p*-hydroxyphenylglycine and β-hydroxytyrosine are derived from tyrosine.

Ristocetin is a glycopeptide antibiotic elaborated by *Nocardia lurida*. It is a member of the vancomycin class of antibiotics known to act by inhibiting bacterial cell wall synthesis.[1] Ristocetin is isolated in two forms, A and B, which differ only by the presence of an additional disaccharide unit in the former. A structure for the ristocetin aglycone, including the stereochemistry at eight of its nine chiral centres, was proposed by Williams and co-workers on the basis of chemical and n.m.r. experiments.[2] Further n.m.r. evidence[3] led to an assignment of the *S* absolute configuration for the remaining chiral centre, at the α-carbon of the *N*-terminal amino acid residue [α1 in (**1**)]. Harris and Harris have recently reported a configurational study of the ristocetin aglycone by chemical degradation and they conclude that this assignment should be reversed.[4] The structure now accepted for the aglycone is that shown in (**1**). A detailed analysis of the ^{13}C n.m.r. spectra of ristocetins A and B has been published[5] and we now present the results of a biosynthetic investigation of the peptide aglycone using ^{13}C labelled precursors. We have elucidated the origins of all the phenolic amino acids of which it is composed. This work has simultaneously led to assignments of some of the carbonyl resonances, which were not previously established.



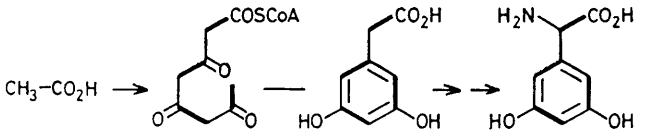
(1)

A fermentation of *N. lurida* (1 l) was supplemented with DL-[α-^{13}C]tyrosine (500 mg; 90 atom % ^{13}C) 22 h after inoculation. After a further 26 h, the culture was harvested and ristocetin isolated as the complex of A and B components. The ^{13}C n.m.r. spectrum at 100.6 MHz of the antibiotic showed that five carbon atoms in the aglycone were enriched with ^{13}C. These were α2, α6, and three whose resonances were in the carbonyl region of the spectrum. By analogy with the result of incorporating the same labelled amino acid into vancomycin,[6]

Table 1. Incorporation of [α-^{13}C]-*p*-HPG into ristocetin.[a]

Precursor	Amount fed/mg	Enrichment[b]		
		α1	α4	α5
DL	150	7.7	5.7	4.8
D	102	14.0	8.1	8.1
L	60	9.3	6.0	5.2

[a] In each experiment 500 ml of fermentation broth was used. The precursor was added 22 h after inoculation of the culture and the antibiotic harvested 26 h later. [b] Enrichment was measured as the factor by which the height of a signal in the ^{1}H-noise decoupled ^{13}C n.m.r. spectrum is increased over its height in a natural abundance spectrum acquired under the same conditions.

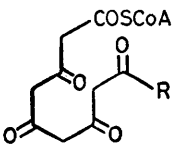


Scheme 1

the three enriched carbonyls are taken to be those of the three *p*-hydroxyphenylglycine (*p*-HPG) residues, X1, X4, and X5. The conversion of tyrosine into *p*-HPG has been illustrated in the biosynthesis of nocardicin A.[7] However, an early labelling experiment on avoparcin,[8] another antibiotic in the vancomycin class, was inconclusive regarding the biogenetic origin of the types of substituted phenylglycine in this class of compounds.

Feeding experiments were carried out using as precursors DL-[α-^{13}C]-*p*-HPG and its separate enantiomers. Incorporation was obtained in all cases with enrichment only at the α-position of each *p*-HPG residue. The results are summarised in Table 1. L- and D-*p*-HPG are incorporated into ristocetin with comparable efficiency. It is likely that racemisation of the amino acid occurs *in vivo*. This contrasts with the biosynthesis of nocardicin A, by *Nocardia uniformis tsuymanensis*, in which only the L-form of *p*-HPG increases production of the metabolite[7a] and is incorporated into it.[7b]

^{13}C-Labelled ristocetin was also obtained from a culture (500 ml) fed with sodium [1,2-$^{13}C_2$]acetate (500 mg; 90 atom % ^{13}C) in two portions 20 h and 28 h after inoculation. The antibiotic was isolated after a total of 48 h. Its ^{1}H-noise decoupled ^{13}C n.m.r. spectrum revealed ^{13}C–^{13}C spin-coupled doublets, flanking the natural-abundance signal, for each sp^2 carbon atom of rings D and F. Furthermore, there were similar doublets for carbons α7 and α3, and for two in the carbonyl region of the spectrum. The latter were assigned as X7 and X3 from their respective coupling constants of 65 and 56 Hz to the α-carbon atoms. The incorporation of acetate into the two *m*,*m*′-dihydroxylated phenylglycine moieties, rings D and F, implies that they can be assembled by the cyclisation of a polyketide. A mode of cyclisation that is consistent with the observed labelling patterns is shown in Scheme 1. It is, however, highly improbable that ring closure occurs onto a terminal methyl group as indicated. The biosynthesis may proceed *via* an intermediate with an additional chain-starting acyl group (**2**) which is lost after formation of the ring.



(2)

Financial support from the S.E.R.C. is gratefully acknowledged.

Received, 10th November 1982; Com. 1288

References

1 D. H. Williams, V. Rajananda, M. P. Williamson, and G. Bojesen, 'Topics in Antibiotic Chemistry,' Vol. 5, ed. P. Sammes, Ellis Horwood, Chichester, 1980, p. 119; H. R. Perkins, *Pharmac. Ther.*, 1982, **16**, 181.

2 D. H. Williams, V. Rajananda, and J. R. Kalman, *J. Chem. Soc., Perkin Trans.* 1, 1979, 787; J. R. Kalman and D. H. Williams, *J. Am. Chem. Soc.*, 1980, **102**, 897.

3 D. H. Williams, V. Rajananda, G. Bojesen, and M. P. Williamson, *J. Chem. Soc., Chem. Commun.*, 1979, 906.

4 C. M. Harris and T. M. Harris, *J. Am. Chem. Soc.*, 1982, **104**, 363.

5 M. P. Williamson and D. H. Williams, *J. Chem. Soc., Perkin Trans.* 1, 1981, 1483.

6 A. Bongini, J. Feeney, M. P. Williamson, and D. H. Williams, *J. Chem. Soc., Perkin Trans.* 2, 1981, 201.

7 (a) J. Hosoda, N. Tani, T. Konomi, S. Ohsawa, H. Aoki, and H. Imanaka, *Agric. Biol. Chem.*, 1977, **41**, 2007; (b) C. A. Townsend and A. M. Brown, *J. Am. Chem. Soc.*, 1981, **103**, 2873.

8 W. J. McGahren, J. H. Martin, G. O. Morton, R. T. Hargreaves, R. A. Leese, F. M. Lovell, G. A. Ellestad, E. O'Brien, and J. S. E. Holker, *J. Am. Chem. Soc.*, 1980, **102**, 1671.